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Environmental biotechnology for hazardous wastes is operationally defined as the use of living organisms or their processes for socio-economic benefit in environmental protection and restoration. Often, biotechnology for control of wastes and toxic materials is viewed as the extremes of either conventional biological waste treatment technology or genetically engineered "super bugs" of consequent risk to the environment. Between these extremes, environmental biotechnology has evolved from the integration of Engineering, Environmental and Biological sciences as an important new research field contributing to the development, application and optimization of biological processes in hazardous waste control. An analysis of applications of biological process in hazardous waste control leads to the identification of major areas in which environmental biotechnology can contribute new problem solutions and directions for the development or more reliable technology.

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ENVIRONMENTAL BIOTECHNOLOGY: MOVING FROM THE FLASK TO THE FIELD

Special Final Report

Grant No. AFOSR-91-0046

August 30, 1991

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1 INTRODUCTION

On October 17-19, 1990, a symposium entitled "Environmental Biotechnology--Moving From the Flask to the Field" was hosted at the University of Tennessee Conference Center in Knoxville, Tennessee. Over 300 individuals attended this meeting to hear and interact with forty Session Chairpersons, Invited Speakers and Reviewers.

Sponsors included International Technology Corporation, American Cyanamid Company and the University of Tennessee Waste Management Research and Education Institute. The U.S. Air Force Office of Scientific Research was a sponsoring agency and the Oak Ridge Waste Management Association was a supporter.

The first symposium of its kind, the theme of the meeting was to merge an understanding of the unique interests of waste generators, environmental service contractors, vendors, regulators, concerned citizens and the research community as they relate to the performance of field bioremediation processes. The perceptions, state-of-the-art research and commercial applications, and both technical and non-technical problems experienced in moving environmental biotechnology from the flask to the field were addressed.

2 PLANNING

The process of planning both the topics and structure of the symposium and the invited speakers began with the actions of the Steering Committee. Members of the Steering Committee included:

Bob Allen	International Technology Corporation
James Blackburn	Energy, Environment, and Resources Center, The University of Tennessee, Knoxville
Bill Colglazier	Waste Management Research and Education Institute, The University of Tennessee, Knoxville
Jimmy Cornette	Environics Division, U.S. Air Force Engineering and Services Center
John Corey	Westinghouse/DOE Savannah River Site
Sue Markland Day	Center for Environmental Biotechnology, The University of Tennessee, Knoxville
Bob Fox	International Technology Corporation
Tom Hayes	Gas Research Institute
Ray Hillard	Lederle Laboratories
Margaret Kelly	Office of Solid Waste and Emergency Response, U.S. Environmental Protection Agency
Maureen Leavitt	International Technology Corporation
Anthony Malinauskas	Martin Marietta Energy Systems/DOE Oak Ridge National Laboratory
Gary Sayler	Center for Environmental Biotechnology, The University of Tennessee, Knoxville
Godfred Tong	Monsanto Company
J. Carroll Duggan	Waste Technology Program,Valley Resource Center, Tennessee Valley Authority
Dennis Wynne	Technical Support Division, U.S. Army THAMA

The Steering Committee appointed James Blackburn the Symposium Coordinator with the responsibility of organizing the program and the sessions accordingly. Elaine Keener of the University of Tennessee Conference Center was appointed Program Manager and together with her staff, Elaine was responsible for the details of organizing and holding the meeting. Gary Sayler was appointed the Proceedings Coordinator and had responsibility for producing a proceedings volume entitled Environmental Biotechnology for Waste Treatment (G. S. Sayler, R. Fox and J. W. Blackburn, eds., Plenum Press, New York, 1991).

The program was divided into sessions and Session Chairs were appointed. The Session Chairs had primary responsibility in contacting, inviting, and scheduling the speakers nominated by the Steering Committee. The Session topics and Chairs are as follow:

Session I-Environmental Biotechnology: Current Perceptions	James Blackburn, The University of Tennessee
Session II-Environmental Biotechnology, Field-scale Case Studies	Jimmy Cornette, Environics Division, Air Force Engineering and Service Center
Session III-Technical Issues and Concerns in Environmental Biotechnology Implementation	Arthur Day, Bechtel Environmental, Inc. and Gene Bowlen, Rutgers University
Session IV-Nontechnical Issues and Concerns in Environmental Biotechnology Implementation	Sue Markland Day, The University of Tennessee and Margaret Kelley, OSWER, U.S. EPA
Session V-International Activities in Environmental Biotechnology	David C. White, The University of Tennessee

Session VI-Symposium Review, Analysis, and Discussion	Robert Goldstein, Electric Power Research Institute

3 PROGRAM

WEDNESDAY, OCTOBER 17, 1990

WELCOME AND OPENING REMARKS Bill Colglazier, Director, Waste Management Research and Education Institute, The University of Tennessee, Knoxville.

SESSION I - Environmental Biotechnology: Current Perceptions

INTRODUCTORY COMMENTS Chair: James Blackburn, Ph.D., Associate Director, Energy, Environment and Resources Center, The University of Tennessee, Knoxville.

ENVIRONMENTAL BIOTECHNOLOGY: PERCEPTIONS, REALITY, AND APPLICATIONS Gary S. Sayler, Ph.D., Director, Center for Environmental Biotechnology, The University of Tennessee, Knoxville and Robert D. Fox, Distinguished Technical Associate, International Technology Corporation.

MEDIA IMAGES OF ENVIRONMENTAL BIOTECHNOLOGY: WHAT DOES THE PUBLIC SEE? Mike R. Fitzgerald, Ph.D., and Amy S. McCabe, Ph.D., Energy, Environment, and Resources Center, University of Tennessee, Knoxville and Vanderbilt University Institute for Public Policy Studies.

PERSPECTIVES ON BIOREMEDIATION IN THE GAS INDUSTRY David G. Linz, Gas Research Institute, Edward F. Neuhauser, Ph.D., Niagara Mohawk Power Company, and Andrew C. Middleton, Ph.D., Remediation Technologies, Inc.

CONSIDERATIONS IN THE SELECTION OF ENVIRONMENTAL BIOTECHNOLOGY AS VIABLE IN FIELD-SCALE WASTE TREATMENT APPLICATIONS Pat Taylor Woodyard, CH2M-Hill, Inc.

THE TECHNICAL, ECONOMIC AND REGULATORY FUTURE FOR BIOREMEDIATION: AN INDUSTRY PERSPECTIVE Keith Kaufman, President, Applied BioTreatment Association and Vice President, Thorne Environmental, Inc.

REMOVING IMPEDIMENTS TO THE USE OF BIOREMEDIATION AND OTHER INNOVATIVE TECHNOLOGIES Walter Kovalick, Ph.D., Director, Technology Innovation Office, U.S. Environmental Protection Agency.

BIOREMEDIATION RESEARCH ISSUES John H. Skinner, Ph.D., Deputy Assistant Administrator, Office of Research and Development, U.S. Environmental Protection Agency.

SESSION II - Environmental Biotechnology Field-Scale Case Studies

INTRODUCTORY COMMENTS Chair: Jimmy Cornette, Ph.D., Senior Scientist, Environics Divison, U.S. Air Force Engineering and Services Center.

BIOREMEDIATION OF THE FRENCH LIMITED SUPERFUND SITE--FEASIBILITY STUDIES TO THE CONSENT DECREE R. E. Woodward, Ph.D., Vice President, Bioremediation and R. K. Ramsden, Ph.D., ENSR Corporation.

EVALUATION OF BIOREMEDIATION IN A COAL COKING WASTE LAGOON Maureen Leavitt, Project Technical Coordinator, International Technology Corporation.

EVALUATION OF COMMERCIAL BIOREMEDIATION IN ALASKA Val Kelmeckis, Director of Technology Evaluation, National Environmental Technology Applications Corporation.

FULL-SCALE REMEDIATION OF CONTAMINATED SOIL AND WATER Geoffrey Compeau, Ph.D. and William D. Mahaffey, Ph.D., ECOVA Corporation. Lori Patras, Unocal Corporation.

Panel Discussion

WHAT WILL THE BIOREMEDIATION BUSINESS BE LIKE IN 1995? Moderator: Edgar Berkey, Ph.D., Executive Vice President, National Environmental Technology Applications Corporation.

THURSDAY, OCTOBER 18. 1990

SESSION III - Technical Issues and Concerns in Environmental Biotechnology Implementation

INTRODUCTORY COMMENTS Co-Chairs, Arthur Day, Technical Integration Manager for ORNL's RI/FS Project, Bechtel Environmental, Inc. and Gene Bowlen, Department of Chemical and Biochemical Engineering, Rutgers University.

FEASIBILITY AND OTHER CONSIDERATIONS FOR USE OF BIOREMEDIATION IN SUB-SURFACE AREAS Karolyn L. Hardaway, Ph.D., Texas Eastman Company

INTEGRATION OF BIOTECHNOLOGY TO WASTE MINIMIZATION PROGRAMS Godfred E. Tong, Ph.D., Manager Process Technology, Corporate Research, Monsanto Company.

BIO-REMEDIATION OF EXPLOSIVES CONTAMINATED SOILS (SCIENTIFIC QUESTIONS/ENGINEERING REALITIES) Capt. Craig A. Myler, Ph.D., Project Officer and Wayne Sisk, U.S. Army Toxic and Hazardous Materials Agency.

PRACTICES, POTENTIAL, AND PITFALLS IN THE APPLICATION OF BIOTECHNOLOGY TO ENVIRONMENTAL PROBLEMS Carol D. Litchfield, Ph.D., Senior Vice President, Environment America, Inc.

WHAT IS THE Km OF DISAPPEARASE? Ronald Unterman, Ph.D., Vice President, Research and Development, Envirogen, Inc.

USE OF TREATABILITY STUDIES IN DEVELOPING STRATEGIES FOR CONTAMINATED SOILS Michael J. McFarland, Ph.D., and Ronald C. Sims, Ph.D., Division of Environmental Engineering, Utah State University, and James W. Blackburn, Ph.D., Associate Director, Energy, Environment and Resources Center, The University of Tennessee, Knoxville.

BIODEGRADATION OF MIXED SOLVENTS BY A STRAIN OF PSEUDOMONAS Jim C. Spain, Ph.D., U.S. Air Force Engineering and Service Center.

SESSION IV - Nontechnical Issues and Concerns in Environmental Biotechnology Implementation

INTRODUCTORY COMMENTS Co-Chairs: Sue Markland Day, Environmental Biotechnology Policy Specialist, The University of Tennessee, Knoxville and Margaret Kelly, Office of Solid Waste and Emergency Response, U.S. Environmental Protection Agency.

THE FIELD IMPLEMENTATION OF BIOREMEDIATION: AN EPA PERSPECTIVE Fran Kremer, Ph.D., Senior Environmental Engineer, Office of Research and Development, U.S. Environmental Protection Agency.

A HISTORICAL PERSPECTIVE: DOES GOOD PRESS AND/OR GOOD SCIENCE GENERATE DEMAND? Thomas Zitrides, President, BioScience Management, Inc. and Vice President, Applied BioTreatment Association.

WAYS TO IDENTIFY AND OBTAIN RIGHTS TO TECHNOLOGY FROM FEDERAL FACILITIES John C. (Jack) Corey, Ph.D., Manager of Technology Transfer, Westinghouse Savannah River Site.

AN OVERVIEW OF CURRENT ATTITUDES ON THE USE OF BIOTREATMENT FOR CLEANUPS William J. Lacy, Dr.Sc., P.E., D.E.E., President, Lacy & Company.

VIEWS OF A PROJECT MANAGER: WHAT ARE THE CRITICAL FACTS NECESSARY TO WIN THE CONFIDENCE OF THE STATE REGULATOR? Frank R. Peduto, P.E., Senior Engineer, New York State Department of Environmental Conservation.

IS THERE A NEED FOR A BIOREMEDIATION SPECIALIST CERTIFICATION? Morris A. Levin, Ph.D., Program for Public Issues in Biotechnology, University of Maryland.

BIOLOGICAL MONITORING TO DEMONSTRATE CLEANUP ACTIONS Carl Gehrs, Ph.D., Environmental Sciences Division, Oak Ridge National Laboratory.

FEDERAL REGULATIONS: HOW THEY IMPACT RESEARCH AND COMMERCIALIZATION OF BIOLOGICAL TREATMENT Sue Markland Day, Center for Environmental Biotechnology, University of Tennessee, Knoxville.

FRIDAY, OCTOBER 19, 1990

SESSION V - International Activities in Environmental Biotechnology

INTRODUCTORY COMMENTS Chair, David C. White, M.D., Ph.D., Distinguished Scientist, The University of Tennessee, Knoxville.

POLLUTED HETEROGENEOUS ENVIRONMENTS: MACRO-SCALE FLUXES, MICRO-SCALE MECHANISMS AND MOLECULAR-SCALE CONTROL Prof. G. Hamer, Ph.D., Institute of Aquatic Science and Water Pollution Control, Swiss Institute of Technology, Zurich, Switzerland.

THE PILOT PLANT TESTING OF THE CONTINUOUS EXTRACTION OF RADIONUCLEIDES USING IMMOBILIZED BIOMASS Marios Tsezos, Ph.D, Department of Chemical Engineering, McMaster University, Hamiliton, Ontario, Canada and Ronald McCredy, Ph.D, Director of Biotechnology, CANMET, Canada.

RESEARCH AND DEVELOPMENT PROGRAM FOR BIOLOGICAL HAZARDOUS WASTE TREATMENT IN THE NETHERLANDS Esther R. Soczo, Coordinator for Development of Remedial Action Technologies for Contaminated Soils and Deputy Chief of the Department of Environmental Technology, National Institute of Public Health and Environmental Protection, RICM, Bilthoven, Netherlands.

SESSION VI - Symposium Review, Analysis, and Discussion

INTRODUCTORY COMMENTS Chair, Robert Goldstein, Program Director, Electric Power Research Institute.

REVIEW, ANALYSIS, AND DISCUSSION Al Bourquin, Ecova Italia; Thomas W. Federle, Proctor and Gamble, Ivorydale Technical Center; C. P. Leslie Grady, Clemson University; and William D. Mahaffey, Ecova Company.

4 ABSTRACTS

4.1 PRESENTATION ABSTRACTS

4.1.1 SESSION I--ENVIRONMENTAL BIOTECHNOLOGY: CURRENT PERCEPTIONS

Environmental Biotechnology: Perceptions, Reality and Applications, Gary S. Sayler, Ph.D., The University of Tennessee, Knoxville and Robert D. Fox, International Technologies Corporation, Knoxville, TN

Environmental biotechnology for hazardous wastes is operationally defined as the use of living organisms or their processes for socio-economic benefit in environmental protection and restoration. Often, biotechnology for control of wastes and toxic materials is viewed as the extremes of either conventional biological waste treatment technology or genetically engineered "super bugs" of consequent risk to the environment. Between these extremes, environmental biotechnology has evolved from the integration of Engineering, Environmental and Biological sciences as an important new research field contributing to the development, application and optimization of biological processes in hazardous waste control. An analysis of applications of biological process in hazardous waste control leads to the identification of major areas in which environmental biotechnology can contribute new problem solutions and directions for the development or more reliable technology.

Media Images of Environmental Biotechnology: What Does the Public See? Michael R. Fitzgerald, Ph.D. and Amy S. McCabe, Ph.D., Energy, Environment, and Resources Center, The University of Tennessee, Knoxville and Vanderbilt University Institute for Public Policy Studies

Environmental biotechnology, as a method for effective and economical treatment of hazardous wastes, is one of the most recent applications of biotechnology to a major societal problem. Research and development in this area continues apace, though fundamental policy questions relating to the successful transfer of technology from laboratories to the private sector remain unanswered.

How, and to what extent the government should regulate environmental biotechnology, and educate and inform the public are critical issues that public policy makers must address.

The national television news plays a key role in biotechnology policy direction and redirection in the United States. By communicating biotechnology information directly to a mass audience, network news can educate viewers about topics where a majority of Americans would not otherwise be attentive. Television news is also influential because of its powerful potential to shape public opinion about biotechnology applications. Finally, by featuring biotechnology stories, news segments often emphasize a critical lack of research support dedicated to conceivably promising technologies. By controlling the flow of information--the context in which it is presented, its position in the broadcast, and the amount of time devoted--the news makes a wide-reaching statement about the relative importance of biotechnology issues.

This presentation examines how environmental biotechnology has been portrayed by major network evening news organizations. Based on a systematic examination of taped newscasts obtained from the Vanderbilt University Television News Archive, the study illustrates how biotechnology has evolved, if and how environmental biotechnology coverage differs from that of other biotechnology applications, and what public perceptions are likely to be fostered by the mass media portrayal of biotechnology issues. Implications for environmental biotechnology policy are given.

Perspectives on Bioremediation in the Gas Industry David G. Linz, Gas Research Institute, Edward F. Neuhauser, Ph.D., Niagara Mohawk Power Corporation, and Andrew C. Middleton, Ph.D., Remediation Technologies, Inc.

While there may be several applications for bioremediation in the gas industry, the one of most immediate interest deals with the cleanup of manufactured gas plant (MGP) sites. These plants operated in the U S. from 1816 to the 1960's, producing manufactured or "town" gas from coal and oil used for lighting, cooking, and some industrial purposes. One of the by-products of gas production was tar and other organic residues which can be currently found in the soil and groundwaters at many MGP sites. Since 1986 the Gas Research Institute (GRI) along with individual gas companies have been sponsoring research focused on improving investigative and remediation methods for MGP sites. Early on it was demonstrated that contaminated groundwater from these sites could be bioremediated in municipal activated sludge systems along with municipal waste-water. The next area of opportunity for bioremediation to be examined was the treatment of soils contaminated with organic residues.

The ideal process for treatment of such soils would be one that consistently and predictably destroys a high percentage of the tar contaminants, i.e. polynuclear aromatic hydrocarbons (PAHs), at a low cost without creating any other adverse by-products. Testing of the treatment technologies has shown that thermal processes, e.g. incineration, consistently and predictably destroy greater than 99% of the PAHs, but are costly. Conversely, testing has also shown that bioremediations may degrade PAHs at a low cost but not consistently and predictably. A key need for the gas industry is a rapid, low-cost means to reliably evaluate the effectiveness of bioremediation for MGP residues. Further, once it can be shown that the residues are treatable using bioremediation, it must be demonstrated that full scale systems can be designed and the performance reliably predicted and controlled.

In numerous bioremediation tests of soils from different MGP sites, results have shown the level of PAH treatment varying from zero to 90% with endpoints for PAH ranging less than 10 to greater than 1000 ppm PAH. An analysis of these tests has shown that soil type appears to be a major factor in these inconsistent results. Soils which yield the higher endpoint concentrations of PAHs are the ones containing higher amounts of clay and organic matter and/or significant amounts of free tar. Bioremediation technology appears to be limited for those wastes which have a high affinity for adsorption on soil particles and perhaps for soils with high clay and/or organic matter.

A testing protocol has been developed to provide more rapid estimation of bioremediation treatment endpoints. It is based in part on desorption isotherms which determine the degree to which contaminants such as PAHs partition to the soil water, and hence become bioavailable. The remaining PAHs are highly bound to the soil matrix and are not bioavailable in bioremediation processes. Consequently, they are also not mobile in the environment and pose substantially lower risks. Thus, although not able to produce consistent and predictable destruction of all PAHs, bioremediation may consistently produce an environmentally acceptable treatment endpoint. Further data on the environmental behavior of these treated residues is needed to convince the gas industry and regulatory agencies of the validity of this approach.

Widespread acceptance among the regulatory community that a bioremediated soil is environmentally acceptable independent of the endpoint concentration of PAHs has not been achieved. Often it is very difficult to obtain a fair consideration of bioremediation with the certainty of the performance of incineration looming in the background even in spite of the substantially higher costs and the hurdles encountered in obtaining air emissions permits. This situation epitomizes one of the greatest limitations to widespread use of bioremediation. Its resolution is essential to the future implementation of bioremediation of soils at MGP sites and most likely at other industrial sites.

The next area of opportunity for bioremediation at MGP sites is the in situ bioremediation of soils and groundwater. This process offers the potential for environmentally sound, cost-effective remediation of deeper contamination making it very attractive to the gas industry. However, significant development and demonstration will be required before it can be widely applied at MGP sites as many technical and regulatory issues remain.

Considerations In The Selection of Environmental Biotechnology as Viable In Field-Scale Waste Treatment Applications Patricia Taylor Woodyard, Industrial Hazardous Waste Services, CH2M Hill, Inc.

The selection of environmental biotechnology for field-scale implementation at contaminated sites results from an identification, evaluation and weighing the impact of numerous issues. This technology's <u>technical</u> viability is often weighed against <u>non-technical</u> issues and the risks associated with its implementation.

The technology is often then compared to issues and risks arising from implementing alternative technologies at field-scale. The non-technical issues under consideration often include the level of uncertainty associated with the technology in combination with legal, regulatory, sociopolitical, and business implications. Any issue or combination of issues can outweigh the potential technical feasibility of environmental biotechnology. Identifying and evaluating the considerations and implications associated with these technical and non-technical issues under site remediation circumstances is a valuable step in determining the viability of environmental biotechnology. "Uncertainty" becomes a key component that can influence both the technical and non-technical evaluations.

The level of uncertainty with biotechnology's application under site specific conditions can affect the selection of this technology. The uncertainty of the technology's technical feasibility, irrespective of its application on a specific site, is compounded by the uncertainty once the technology is applied to site specific subsurface conditions. These uncertainties can in turn affect the viability of biotechnology's application on a site when combined with other technical and non-technical issues. This paper will describe these issues and associated considerations that can affect the application of environmental biotechnology under field-scale conditions.

The Technical, Economic, and Regulatory Future for Bioremediation: An Industry Perspective A. Keith Kaufman, M.S., BIOTA Division, Thorne Environmental, Inc., and Applied Bio-Treatment Association

The Biotreatment industry has undergone major evolutionary changes over the last decade. From a little known, poorly understood approach to environmental cleanup, bioremediation has rapidly developed into a widely accepted form of remedial technology for a variety of environmental pollutants. Whereas the number of companies offering biotreatment in various forms five years ago could be counted on two hands, current estimates indicate that nearly 200 such companies now operate in the United States alone. Much of this rapid growth is due to increased reports of successful bioremedial cleanups, as well as an increased understanding among industry, regulators, and the public of the operational principles associated with the technology.

While hope remains for continued growth and utilization of biotreatment, many issues which could preclude the economic as well as practical benefits associated with the technology currently face the industry. So critical are these issues that unless strong countermeasures are adopted and implemented, the survival of the biotreatment industry will be in serious jeopardy.

This presentation will focus on those issues which the industry believes pose the greatest threat to the continued development and utilization of bioremedial technology. Major emphasis will be placed on federal regulatory constraints which could undermine the very technology EPA has publicly supported. The significant economic and technical implications of these regulatory developments will also be explored. Efforts presently underway by the biotreatment industry to mitigate these issues will also be addressed, as will developing criteria for the registration of certified biotreatment specialists, a move designed to build industry credibility by documenting bioremedial competency of vendor companies.

Removing Impediments to the Use of Bioremediation and Other Innovative Technologies Walter W. Kovalick Jr., Ph.D., Technology Innovation Office, Office of Solid Waste and Emergency Response, U.S. Environmental Protection Agency

The Office of Solid Waste and Emergency Response has set as one of its goals increased diversity of technologies used to remediate contaminated soils and ground water. There are statutory and economic reasons to move away from conventional methods such as stabilization, containment and incineration. Impediments exist however, to the application of any innovative technology. These include inhibiting regulations, conservative attitudes and fear of risk, and lack of information on performance and cost. The Agency is addressing these impediments through several avenues. Bioremediation is of special interest because of the potential for cost-effectiveness and permanence compared to historical technologies.

We are pursuing changes in the implementation of the Resource Conservation and Recovery Act (RCRA) to reduce obstacles to the use of innovative technologies at Corrective Action sites and Superfund sites. Policy changes are also underway to make it acceptable to "fail" during the first applications of a new technology. Finally, we are making efforts to increase our knowledge of performance and cost through a Bioremediation Field Initiative -- an evaluation of actual treatment systems now operating at sites.

Bioremediation Research Issues John H. Skinner, Ph.D, Research and Development, U.S. Environmental Protection Agency

Biological treatment is an effective remedial technology for managing liquid and solid hazardous wastes in above-ground reactors and in-situ systems. Over the past several years EPA has had a number of successful bioremediation applications to emergency responses such as oil contaminated beach clean ups from spills, and Superfund remedial action clean-ups for sludges containing polychlorinated biphenyls, volatile organics and metals, as well as soils contaminated with creosote and poly aromatic hydrocarbons.

On February 22, 1990, EPA conducted a meeting on the Environmental Applications of Biotechnology. Administrator Reilly and four EPA Assistant Administrators, as well as close to 150 senior representatives from Federal/state government, industry and academia attended the one day workshop. The purpose of the meeting was to prepare a biotechnology agenda for action for the 1990's. Most of the discussion at this meeting had to do with bioremediation. The findings, recommendations, and proposed actions from the EPA-Industry meeting were divided into six areas: field tests and demonstrations, technology transfer, education and training, policy and regulations, research and pollution prevention.

A joint Federal agency, academic and industry committee was established to implement the actions from the February meeting. Some examples of these actions include:

Field Test and Demonstrations -

- EPA is developing guidance for bioremediation treatability tests and demonstrations.

- EPA will work with industry to conduct additional field tests of bioremediation at Superfund, RCRA corrective action and underground storage tank sites.

- Working jointly with the State of Alaska and Exxon, EPA plans to coordinate additional bioremediation field tests in Prince William Sound, Alaska on biological oil degradation on contaminated beaches.

Policy and Regulations -

- EPA's Office of Solid Waste and Emergency Response is considering a more proactive policy statement regarding the use of innovative technologies (including bioremediation) under Superfund as well as its other programs.

- EPA may be asking for additional bioremediation performance data for the proposed Land Disposal Restriction Regulations for soil and debris.

- States may be establishing bioremediation requirements for clean up of state lead hazardous waste sites.

Research -

- EPA is developing a research initiative for 1992 that will expand its existing research program to address critical gaps in the science of bioremediation.

- The critical role of academic Institutions in conducting research on bioremediation will be reviewed and may be expanded.

4.1.2 SESSION II--ENVIRONMENTAL BIOTECHNOLOGY: FIELD CASE STUDIES

Bioremediation of the French Limited Superfund Site - Feasibility Studies to the Consent Decree R.E. Woodward, Ph.D. and D.K. Ramsden, ENSR Consulting and Engineering

In situ bioremediation of mixed, hazardous waste was demonstrated in a 0.5 acre portion of the 7.3 acre lagoon. The petrochemical and other industrial waste sludges contained volatiles, polynuclear aromatic hydrocarbons, chlorinated solvents, and PCB priority pollutants in a viscous, hydrophobic, tarry matrix under 3 to 15 feet of water. The growth and metabolic activity of microorganisms native to the site was stimulated by a four phased mixing approach and by providing a suitable pH, essential nutrients and oxygen. Priority pollutant concentrations, wastewater treatment parameters and toxicity were monitored to control operations and to document the progress of bioremediation.

The demonstration confirmed the feasibility of in situ bioremediation and led to one of the first U.S. EPA Record of Decisions to use in situ bioremediation for cleanup of a large superfund site. A consent decree outlining the site remedial action plan was signed by the PRP task group and published in the federal register. This represents a landmark project for in situ bioremediation and has established precedence for use of this technology at CERCLA and RCRA sites nationwide.

Evaluation of Bioremediation in A Coal-Coking Waste Lagoon M.E. Leavitt, D.A. Graves, Ph.D., and C.A. Lang, International Technology Corporation

A remedial investigation/feasibility study is being completed for a former coal coking plant under the Superfund program. Bioremediation is one of the many alternatives being evaluated as remedial tools for the waste lagoons. The lagoons contain elevated concentrations of coal, metals, cyanide, phenolics and polyaromatic hydrocarbons. A bench and pilot-scale study was designed and executed to determine the overall effect of bioremediation on the organic contaminants and to collect the data to determine the overall feasibility of implementing this technology in a full-scale system.

The bench-scale testing included a respirometer study using 10:1 and 50:1 ground water:soil slurries. The results indicated that an abundant population of viable organisms were capable of degrading the organic carbon present in the soil and water. The biologically-active slurries exhibited greater that 99% reduction in the specific organic contaminants.

The pilot study included: a) an in situ subsurface remediation system using a center injection well and perimeter recovery wells, b) sprinkler nutrient injection system to treat the unsaturated zone. Injection water was supplemented with nutrients and hydrogen peroxide (for the subsurface treatment) for six months of continual operation. Physical, biological and contaminant data were collected on a weekly and monthly basis.

The physical and chemical data indicated that oxygen and nutrients were being effectively transported throughout each system. Microbial populations modestly increased, and on occasion have decreased. Contaminant data indicated that total petroleum hydrocarbons, polyaromatic hydrocarbons and phenolics have declined, however, significant variability exists.

The pilot study is scheduled to continue for an additional twelve months, and the data will be used to determine the final record of decision for remediation of these lagoons.

Evaluation Process For The Selection of Bioremediation Technologies For Exxon Valdez Oil Spill Val J. Kelmeckis, NETAC

In the early cleanup stages of the Valdez spill, EPA and EXXON recommended that two bioremediation products should be applied to supplement other more traditional cleaning methods. These two products are fertilizers that enhance the action of naturally occurring microorganisms. Several weeks after application, the beaches where they were used appeared cleaner than control beaches nearby. However, the results were difficult to verify scientifically in the field.

Soon after the spill, pressure began to build to allow other private sector bioremediation companies to use their products in Alaska. The EPA and Coast Guard had received many proposals.

NETAC

In November 1989, EPA asked the National Environmental Technology Applications Corporation (NETAC) to establish criteria by which bioremediation products for cleaning up oil spills could be judged. The criteria were to be practical and consider oil spills in general as well as the Exxon Valdez Spill. NETAC assembled an independent panel of expert scientists from industry, academia, and applied research organizations. The panel developed a set of criteria for evaluation how bioremediation technologies for oil spill cleanup could be chosen.

Next, EPA requested proposals, through an announcement in the Commerce Business Daily outlining proposal criteria developed by the NETAC panel, for bioremediation products that could be used to clean the contaminated beaches. Thirty-nine world-wide proposals were submitted for review.

NETAC reconvened the panel in March 1990 to review the proposals. Ten bioremediation technologies were recommended to EPA for laboratory testing. The purpose of this laboratory evaluation was to further qualify the technologies for possible use on the remnants of the EXXON Valdez oil spill in Alaska during the Summer of 1990.

In June 1990, the NETAC panel reviewed the results of the laboratory tests on the ten technologies and recommended two to EPA for field testing on the weathered crude oil in Prince William Sound. Both products contain naturally occurring microbial cultures with the addition of fertilizer. A field test protocol developed by the EPA and reviewed by NETAC's expert panel was submitted to the agencies responsible for cleanup of the Alaskan spill. Field tests of the two products has been completed with results of the test expected by the end of 1990.

NETAC has established criteria that leave the agencies better prepared to review future bioremediation proposals. EPA plans to institutionalize the lessons learned under this process for future marine oil spills. Protocols have been established for alternative bioremediation technologies to be considered in the future.

Full-scale Bioremediation of Contaminated Soil and Water Geoffrey Compeau, Ph.D. and William D. Mahaffey, Ph.D., ECOVA Corporation and Lori Patras, Unocal Corporation

Biological processes have been used on a large scale to remediate petroleum hydrocarbons, pesticides, chlorinated solvents, and halogenated aromatic hydrocarbons. Biological treatment of contaminated soils may involve solid-phase, slurry-phase, or in situ treatment techniques. This paper will review the general principles of bioremediation and discuss the application of these techniques for full scale cleanup.

Up to 280,000 cubic yards of soil on the site of a former oil refinery tank farm is contaminated with up to 15,000 parts per million (ppm) of petroleum hydrocarbons. The site posed significant challenges due to its size as well as depth and range of contamination. The implementation of biological remediation required the design of a Land Treatment Unit (LTU) and a remedial program which would support the treatment of a significant amount of contaminated soil within a restrictive time schedule. Once this scenario was developed, the LTU was prepared for treatment and excavation and placement of soils began. Currently, the LTU area encompasses 27 acres of a 45 acre site.

A mobile laboratory has been placed onsite and is staffed with chemists and microbiologists who analyze up to 100 soil samples per day. This lab has been designed and equipped to provide the necessary chemical and biological analysis to fully support the excavation and bioremediation program. Onsite biological treatment activities include irrigating, aerating, and tilling the soil to bring microorganisms, contaminants, and oxygen into contact with each other to promote biological degradation. Chemical and microbiological monitoring conducted throughout the remediation process ensures that treatment levels are being met. In addition, numerous site studies were conducted in attempts to define and enhance the remediation process.

A solid-phase bioremediation program was successfully completed at a former wood treating facility; the objective was to biotreat approximately 17,000 cubic yards of soil contaminated with petroleum products, pentachlorophenol, and poly nuclear aromatic compounds.

The bioremediation program consisted of the construction of an onsite soil treatment facility, operation of the facility for approximately six months, and site restoration. Due to the treatment area size, the contaminated soil required treatment in two layers or lifts. Baseline, verification, and clearance sampling and analysis was conducted for each of the two lifts by samples collected and delivered to the ECOVA project laboratory. Treatment operations consisted of daily aeration of the upper lift soils with the addition of water and nutrients to promote and maintain optimum microbial activity and maintain critical remediation parameters. This data and the logistics of this full-scale remediation will be compared to another site containing PCP in soils that was approached through a remediation scheme involving soil washing and aqueous-phase biotreatment.

4.1.3 SESSION III--TECHNICAL ISSUES AND CONCERNS IN ENVIRONMENTAL BIOTECHNOLOGY IMPLEMENTATION

Feasibility and Other Considerations for Use of Bioremediation in Subsurface Areas Karolyn L. Hardaway, Ph.D., Texas Eastman Company

Texas Eastman Company, a manufacturer of chemicals and plastics, is using a pump and treat system to remediate hydrocarbons at a site. In situ bioremediation of the subsurface area is being investigated as a more effective technology to clean the site and to reduce potential environmental impacts. To verify the efficacy of the technology for this application, two laboratory studies were performed. In the first study, laboratory analyses characterized the groundwater and soils from field borings to define factors that would enhance, complicate, or preclude in situ biodegradation. Most data were favorable for bioremediation. Low numbers of microbes in the saturated soils were offset by the large population of microbes contained in the groundwater. Necessary oxygen for aerobic biodegradation could be supplied using hydrogen peroxide since it was not completely oxidized by soil or water samples. Results of nutrient addition and hydrogeological evaluation indicated that soil characteristics in most areas were amenable to in situ biodegradation. The second phase, a bench-scale treatability study, was completed to establish criteria for a bioremediation field trial and determine degradation rates. The criteria developed for the field trial is currently being evaluated. This treatability study indicated that in situ bioremediation in either aerobic or denitrifying atmosphere is a viable method of reducing the hydrocarbon content of the site.

Integration of Biotechnology to Waste Minimization Programs Godfred E. Tong, Ph.D., Monsanto Co.

Chemical engineers need to view biotechnology as one of three treatment technologies available for use in waste minimization projects. As basic understanding on the behavior of industrial biological catalysts and bioreactors emerge, biotechnology needs to be evaluated along with physical and chemical technologies to determine its role in an integrated approach to the design of cost-effective and reliable waste minimization systems.

This presentation provides four different levels for the evaluation of the potential role of biotechnology in waste minimization projects. These four levels are as follows:

1. Biotechnology as a treatment alternative to physical and/or chemical treatment.
2. Biotechnology as a complementary tool either pre or post-treatment to physio-chemical treatment technologies.
3. Biotechnology as a model to arrive at "biomimic" non-living systems approach.
4. Biotechnology as a source of analytical monitoring tool of pollution problems.

Industrial examples for each of the potential roles of biotechnology will be made in this presentation.

Bio-Remediation of Explosives Contaminated Soils (Scientific Questions/Engineering Realities) Craig A. Myler , Ph. D. and Wayne Sisk, U.S. Army Toxic and Hazardous Materials Agency

The explosives trinitrotoluene (TNT), hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) and 1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) have found almost exclusive use as military compounds. As such, the liability for the wastes from past production and processing of these compounds resides, in almost all cases, with the federal government. As the total volume of soils contaminated with these wastes may be substantial, and current remediation costs are high, considerable effort is underway to develop cost effective alternatives for clean-up. Biological methods offer the greatest potential for achieving this goal in the near term. While extensive research has been conducted on the metabolism and environmental fate of these compounds, little information is available for the design of systems capable of exploiting the natural process of utilization. Seeming conflicts between laboratory data and pilot and field scale studies makes implementation difficult. A discussion is presented on the current state of the art in explosives contaminated soil remediation by biological means. Solid phase treatment by composting, aqueous phase treatment by slurry reactor, and inoculation using prepared cultures will be presented. Problems in fielding these methods will also be described. These include methods and frequency of sampling and analysis, final product disposition and fate of the explosives, safety and handling constraints and the costs associated with implementation.

Practices, Potential, and Pitfalls in the Application of Biotechnology to Environmental Problems Carol D. Litchfield, Ph.D., Environment America, Inc.

The use of microorganisms to remediate wastes is not a new technology. In fact, it predates not just modern biotechnology but even the recognition of microorganisms. Composting, and even waste water treatment plants, have been around for a very long time. What is new is the recognition that the concepts embodied in these "ancient" techniques can be applied to the cleaning up of modern contaminated groundwaters, soils, and even process streams. In the rush to embrace this "new" technology, many have forgotten that there have been limitations to composting and waste water treatment systems, and many of these same limitations will apply in the newer applications. Nevertheless, it is still appropriate to examine how biotechnology is being utilized today to solve some of the current environmental problems such as groundwater and aquifer contamination, cyanide leachate from ore extractions, and landfill leachate. We will also examine some of the exciting potentials for the application of biotechnology to such developing areas as waste minimization and agricultural pest control, while remaining mindful of currently known or perceived limitations to the application of biotechnology to solving environmental problems.

What is the K_m of Disappearase? Ronald Unterman, Ph.D., Envirogen, Inc.

It is critically important that throughout biodegradation process development research (and ultimately commercial marketing), investigators clearly demonstrate that their bacterial soil decontamination results are unequivocally due to biological activity. Too often, the results of some studies have not been able to quantitatively account for the disappearance of the target substrate. In some cases, highly hydrophobic contaminants are redistributed in reactors or sorbed to unsampled locations in these reactors. In other studies with, for example, volatile organics, vigorous aeration has potentially volatilized the target instead of biodegrading it. Therefore, biodegradation studies and demonstrations must be designed to determine as best as possible a mass balance of the target substrate and hopefully demonstrate the products of this transformation (ideally, carbon dioxide and water).

One such pitfall that can be encountered in these biodegradation studies can be illustrated by research that the author and his co-workers have been conducting on the biodegradation of PCBs. It is possible that an observed PCB congener depletion in a "biodegradation" process is actually due to physical loss of the PCB and not the true biological degradation. With Aroclor studies, these processes can easily be distinguished because biodegradation results in depletion of specific congeners yielding GC profiles that are distinctly different from those of the original Aroclor mixtures. Physical depletion, on the other hand, results in uniform depletion of all congeners (e.g., adsorptive loss) or depletion of lower chlorinated congeners due to their higher volatility (e.g., evaporative loss). The production of PCB metabolites is, of course, another unequivocal method for demonstrating the biological basis of PCB depletion.

In an attempt to distinguish these different depletion mechanisms and to demonstrate this pitfall, we established a mock, nonbiological "biodegradation" process. PCB-contaminated soil was incubated with stirring in the absence of bacterial inoculum and with a constant stream of inert gas (argon). Following 19 days of incubation, this reactor was analyzed for PCBs in all locations. Although the samples taken from the middle of the soil slurry throughout the 19 days did show greater than 90% depletion of PCBs, the final mass balance was able to account for all of the PCB in the reactor by analyzing the physical components of the reactor (glass walls, stirrer, etc.) as well as coalesced PCB droplets in the bottom of the reactor.

Other studies with volatile organic compounds (e.g., TCE) have required that this research be done in sealed systems. A complete mass balance was therefore determined relative to sterile controls. Such studies, when done under these conditions, do clearly demonstrate that compounds such as TCE can be biodegraded and not volatilized. However, in the final configuration of full-scale bioreactors, the control of aeration will be a critical parameter, as well as monitoring of the bioreactor offgas.

In summary, it is imperative that for bioremediation to be accepted as a safe, cost-effective technology, researchers must unequivocally demonstrate that the loss of the target substrate is indeed due to biological activity and not some other, non-biodegradative effect.

Use of Treatability Studies in Developing Remediation Strategies for Contaminated Soils Michael J. McFarland, Ph.D., Ronald C. Sims, Ph.D., and Division of Environmental Engineering, Utah State University and James W. Blackburn, Ph.D., Energy, Environment and Resource Center, The University of Tennessee, Knoxville

Misunderstanding and confusion regarding use and interpretation of treatability study data has led to difficulty in relating treatability study results to actual performance in field applications. Much of the difficulty stems from misconceptions concerning the objectives of treatability studies, inadequate experimental design, and misapplications of treatability study results for subsurface remediation.

Treatability studies are used to provide specific information concerning chemical mass balances for a waste/soil mixture. By applying the chemical mass balance in treatability studies, the distribution of contaminants among subsurface phases (e.g., gas, water, oil, and soil) can be characterized and used as a basis for treatment technology evaluation and selection. They may also be used to evaluate potential application of treatment technologies at field scale by evaluating and comparing rate and extent of remediation among several technologies. Recognition of uncertainties in the data, for example the use of confidence limits on degradation rates and partition coefficients, represents important information for making decisions at field scale.

Information obtained from treatability studies, conducted as mass balance studies including laboratory screening, bench and pilot-scale studies, can be combined with information concerning site and waste characteristics in order to determine applications and limitations of each potential remediation technology. Results of treatability studies and site characterization data can be used in simulations (e.g., mathematical modeling) in order to: (1) determine containment requirements to prevent contamination of off-site receiver systems; (2) develop techniques to maximize mass transfer of chemicals affecting microorganisms activity; and (3) design a cost-effective and efficient monitoring program to evaluate effectiveness of treatment at field scale.

Biodegradation of Mixed Solvents by a Strain of Pseudomonas J.C. Spain, Ph.D., Air Force Engineering and Services Laboratory

Mixtures of aromatic solvents are poorly degraded by bacteria because most strains can attack only a few closely related chemicals. In addition, simultaneous degradation of alkyl- and chloro-substituted compounds is precluded by misrouting of intermediates and the resulting inhibition of enzymes. The use of mixed cultures can overcome such problems but mixed cultures respond very slowly to changes in substrate feed. Pseudomonas sp. strain JS150 grows on a wide range of substituted aromatic compounds including: benzene, toluene, chlorobenzenes, phenols, and naphthalenes. It also degrades alkyl- and chloro-substituted compounds simultaneously via a modified "ortho" ring-fission pathway. Bench-scale experiments in stirred bioreactors indicate that strain JS150 completely degrades complex mixtures of aromatic solvents added directly to the bioreactors without prior dilution. Chlorobenzene or dichlorobenzene served as the most effective inducers and toluene was the least effective. Cells induced with chlorobenzene cometabolized several compounds including TCE, chlorophenols, and cresols. Absence of metabolites in the culture fluid suggested that these compounds were mineralized even though they could not serve as inducers or as sole sources of carbon. The results indicate that pure cultures of bacteria can be useful for biodegradation of complex mixtures of hazardous wastes.

4.1.4 SESSION IV--NONTECHNICAL ISSUES AND CONCERNS IN ENVIRONMENTAL BIOTECHNOLOGY IMPLEMENTATION

The Field Implementation of Bioremediation: An EPA Perspective Fran Kremer, Ph.D., Office of Research and Development, U.S. Environmental Protection Agency

This past February, the EPA Administrator called a meeting with over 70 representatives from biotreatment companies, site cleanup contractors, industry, academia, environmental organizations, EPA and other Federal agencies. This meeting was designed to identify strategies to increase the use of bioremediation for the cleanup of hazardous wastes.

One of the major themes highlighted in that meeting was the need to expand our field experience using this technology. Even though bioremediation is a viable technology to treat some hazardous wastes, it has not been fully utilized for the many different types of sites requiring remediation. Solid performance data is needed to document the capabilities of this technology. It was recommended that the Agency serve as a focal point in fostering field tests, demonstrations and evaluations of bioremediation, using good test protocols and documentation of results.

Based on this recommendation, the Office of Solid Waste and Emergency Response (OSWER) and the Office of Research and Development (ORD) have instituted a Bioremediation Field Initiative using sites where bioremediation is planned or in progress. This initiative provides assistance to the Regions and states in conducting field tests and carrying out evaluations of site cleanups using bioremediation. Sites considered in this field initiative include CERCLA, RCRA corrective action facilities and UST sites. This initiative is designed to 1) more fully document performance of full-scale field applications of bioremediation, 2) provide technical assistance for sites in a feasibility or design stage to facilitate the conduct of treatability studies, field pilot studies, etc., and 3) regularly provide information on treatability studies, design and full-scale operations of bioremediation projects. This Program is intended to provide information on the operation of biological treatment systems for a variety of wastes and contaminated matrices and provide current cost and performance data.

A Historical Perspective: Does Good Press and/Or Good Science Generate Demand? Thomas G. Zitrides, Bioscience Management, Inc.

Public perception of microorganisms, after Pasteur, has involved a dichotomy between harmful and beneficial "germs". On the one hand, various common phobias, e.g., the Andromeda Strain; on the other, especially in the environmental engineering community, blind reliance that naturally-occuring microorganisms will "just be there" to dispose of waste products, e.g., to treat sewage and industrial wastes (of the "ubiquity principle"). As a result, both regulators and environmental professionals have been slow to accept bioremediation, either with or without exogenous naturally-occuring microbes. These perceptions are reflected repeatedly in the press, in spite of the documented effectiveness of bioremediation techniques.

Regulators are influenced by public phobias as exemplified by the initial ruling out of the use of "non-indigenous, naturally-occuring" microbes to speed degradation of shoreline deposits after the Valdez spill. Compounding the problem are misconceptions of genetic engineering and its confusion with commonly-used accepted techniques of selective adaptation of microbial consortia. The media have both fanned public fears and helped overall bioremediation. Sensationalism, the lack of follow-up, lack of technical competence, and the fairness doctrine which involved obtaining a contrary opinion on virtually any report, have contributed to the problem.

We are now at the peak of the typical 5-year cycle of press interest in bioremediation. A concerted educational effort is required by the bioremediation industry to maintain interest and acceptance. Neither regulators, the press, nor the general public have sufficient information at present to evaluate bioremediation programs realistically.

Ways to Identify and Obtain Rights to Technology From Federal Facilities

John C. Corey, Ph.D., Westinghouse/Department of Energy

The federal government is a storehouse of useful and valuable research information. In the past, American industry has felt that obtaining access to this information was both difficult and non-rewarding. Congress recognized this legitimate concern of the citizens. To correct these shortcomings, Congress passed a series of acts in the 1980's to facilitate the movement of technology to the private sector including the Bayh-Dole Act of 1980, the Stevenson-Wydler Act of 1980, the Technology Transfer Act of 1986 and the National Competitiveness Technology Transfer Act of 1990. As improvements are identified in these laws, additional acts will be forthcoming.

The importance of these acts to the biotechnology arena is that a major focus in the 1990's at many of the federal agencies is environmental restoration and waste management. The federal government will be utilizing major research capabilities to identify superior methodologies to comply with the desires of our citizens to enjoy a safe and clean environment. The technology transfer acts encourage industry to access the unique capabilities at federal facilities. Some of the methods include cooperative research and development agreements between industry and government, personnel exchange, research and development, contracts, patent licensing, small business innovation research program, consulting by laboratory scientists, and technical documents and software.

To facilitate the interchange and to provide a focal point for entry to a specific federal facility, all major research facilities have an Office of Research and Technology Applications (ORTA). The ORTA office acts as a clearinghouse for inquiries, oversees patent licensing, facility use agreements and is the appropriate place to access a particular facility. For more general inquiries there is the Federal Laboratory Consortium (FLC). The FLC represents all federal agencies with active research activities including the Department of Defense, Environmental Protection Agency, NASA, Department of Energy, National Institutes of Health, Department of Agriculture, and others. The FLC is an excellent point to enter the entire federal research establishment when specific knowledge of where the information resides is lacking.

An Overview of Current Attitudes on the Use of Biotreatment For Cleanup
William J. Lacy, Dr.Sc., P.E., D.E.E., Lacy & Company

This paper covers how EPA and industry are emphasizing biotechnology solutions for the treatment of hazardous wastes. Also, to be mentioned are the integrated research, development and demonstration program plans on biosystem technology currently proposed by EPA.

Also, the Hazardous Waste Treatment Council's report which faults EPA's 1988 decisions for the use of bioremediation will be touched upon.

The projections based on the current market for biological treatment of hazardous waste is estimated, primarily on industrial sites and for industrial waste waters.

Mentioned in the paper are a few of the significant institutional barriers to biotechnology and the need to overcome these obstacles as well as the need for the U.S. to either take the lead in the near term or increase our imports from foreign competitors.

Views of a Project Manager: What are the Critical Facts Necessary to Win the Confidence of the State Regulator? Frank R. Peduto, P.E., New York State Department of Environmental Conservation, Bureau of Spill Prevention and Response

Bioremediation has been among the few technologies which have demonstrated the ability to achieve regulatory levels. While this technology has demonstrated a good deal of success, it is not without its drawbacks. Among these are high up-front costs for design, installation and costly nutrient supply throughout the extent of the project. In order to justify this investment, there must be reasonable assurances that it will work. The length of the project and the degree of treatment are typically the least specified items. The regulator needs to be assured of the degree of treatment which will occur and how long will it take. The regulator must be satisfied that all potential negative impacts of this process have been accounted for. They must understand and appreciate the limitations of the process.

This paper will analyze a bioremediation project from two points of view: that of the regulator who has an interest in achieving an environmentally sound solution; and that of a contractor representing a responsible party who in turn have a significant liability and economic interest.

It will discuss the type of information the contractor should be able to provide the regulator to satisfy his/her concerns and to assure a successful completion of the project. It will also identify what issues the regulator must be prepared to provide the contractor in order that a proper estimate and design can be achieved. The establishment of project specifications will assure both parties of what is expected of each other and will most accurately predict the final outcome of the project.

Is There a Need for a Bioremediation Specialist Certification? Morris Levin, Ph.D., University of Maryland, Center for Public Issues in Biotechnology

The issues associated with waste disposal have been identified and described in many places by observers from many perspectives. Legal, safety, technical, environmental, ethical, economic, and socio-political issues have been described in depth over the past decades as public awareness increased of the magnitude of the waste disposal problem. Each waste site or new treatment type raises some or all of the same problems.

As the science and technology associated with waste disposal develops, the technical skills required to select and apply the appropriate remedial action become more critical to the success of the project. In addition, knowledge of applicable statutes, both federal and local, community issues and the potential for long term environmental and public health problems of both the waste and the treatment method is required before appropriate disposal can be achieved.

At present, private companies or municipalities operate waste disposal sites and develop waste disposal procedures and regulatory agencies review and monitor most, but not all, waste disposal practices. Both generally rely on inhouse staff or consultants for technical expertise. Often, however, the expertise is not selected based on objective criteria but on availability of personnel, resulting in costly errors and delays. The result is increased cost, higher probability of environmental damage, and greater potential for adverse health effects.

Many professional groups--ranging from pathologists to midwives to ecologists--have instituted or considered instituting a certification or accreditation program which will assure that a supply of competent specialists will develop. As will be discussed, the basis for these considerations is a recognized need for public confidence in a profession or industry, an understanding that the area of endeavor is becoming highly complex and thus requiring special skills or training, and an interest in minimizing liability for the practitioner.

Many of these arguments are valid for biotechnology as it applies to bioremediation. This paper will examine some of the issues involved in and define the potential need for and value of a certification or accreditation program to the bioremediation industry at the individual and corporate level.

Biological Monitoring Related to Demonstration of Waste Remediation C.W. Gehrs, Ph.D., J.M. Loar, Ph.D., A.J. Stewart, Ph.D., J.F. McCarthy, Ph.D., L.R. Shugart, Ph.D., and S.M. Adams, Ph.D., Environmental Sciences Division, Oak Ridge National Laboratory

Techniques developed through various subfields of environmental biotechnology will play major roles in remediation of waste sites at various Department of Energy facilities. One such component is associated with biological monitoring. This presentation focuses on the experience at Oak Ridge Reservation during the past five years, drawing on specific examples of biological monitoring that have demonstrated the efficacy of remediation activities, aided in identification and prioritization of waste sites, or developed new biochemical techniques for assessing the status of the environment and developing exposure assessments for health risk assessments. Three components will be discussed: environmental toxicology, in-stream monitoring, and biological markers.

Environmental toxicology can serve to identify and prioritize contaminants requiring removal or treatment. It consists of a standard set of biological systems which provide real-time analytical evaluation often more sensitive than chemical analyses. The use of in-stream monitoring to assess the status of the environment, in which the wastes are found or released, determine the potential exposure pathways (for man), identify potential treatment strategies and evaluate is a subfield of growing importance to developing waste management strategies. Biomarkers, for example, refers to any of a series of biochemical or molecular indicators, that can be measured in plants and/or animals on waste sites or through laboratory experiments with wastes treated through various scenarios. The organisms integrate temporally and spatially real world exposures to contaminants developing exposure assessments for human health risks assessments as well as data for environmental risk assessment.

Federal Regulations: How they Impact Research and Commercialization of Biological Treatment Sue Markland Day, Waste Management Research and Education Institute, Center for Environmental Biotechnology, The University of Tennessee, Knoxville

Biology-based waste treatment is a creature of this nation's environmental protection laws. Without the Superfund; water, solid and hazardous waste management programs; and the requirement for leaking underground storage tank cleanup, little research would be underway and little commercial demand would exist for bioremediation. The choice of specific treatment technologies is, to a large part, controlled by laws, regulations, and policies. Such governmental documents may encourage the uses of treatment or may regulate the treatment methodologies (place constraints on the uses of the technology). Often these two conflicting purposes are contained in the same statute.

This speech will introduce the audience to important federal environmental regulations and actions. analyzing each for their positive and negative impacts of bioremediation and new market creation specific to biology-based waste management. After a presentation of specific key federal legislative and regulatory requirements important to bioremediation, the talk will conclude with an analysis of future regulatory trends as they impact environmental biotechnology research and commercial applications.

Six factors, which will be discussed in the speech, promise to contribute to the evolving biological treatment regulatory structure:

(1) The continued debate over the use of technology standards versus the use of health or risk-based standards as treatment objectives.

(2) The high cost of treatment technologies, such as incineration, compared to the anticipated lower cost of bioremediation.

(3) The "Not In My Backyard" syndrome which is encouraging on-site hazardous waste treatment.

(4) An increase in funding for environmental biotechnology research.

(5) An anticipated rapid transition from basic research on environmental biotechnology to in-the-field applications.

(6) The public debate over safety issues associated with the planned release of genetically-altered microbes.

4.1.5 SESSION V--INTERNATIONAL ACTIVITIES IN ENVIRONMENTAL BIO-TECHNOLOGY

Polluted Heterogeneous Environments: Macro-scale Fluxes, Micro-scale Mechanisms and Molecular-scale Control Geoffrey Hamer Ph.D., Institute for Aquatic Sciences and Water Pollution, Swiss Federal Institute of Technology, Zurich, Switzerland

Until some 10 years ago, microbiological research was dominated by an over-emphasis on both pure monocultures and single carbon energy substrates, on the one hand, and by the assumptions that most cultures are essentially homogeneous and that any micro-scale heterogeneities were largely irrelevant to culture performance, on the other hand. Whilst clearly, many important discoveries were made within the scope of these limitations, today's pressing problems for effective, efficient and economic microbially mediated remediation technologies for dangerously polluted environments require an entirely different approach. The major question is how can microbial consortia be harnessed for accelerated rate bioremediated processes in markedly heterogeneous, seriously perturbed natural and/or inappropriately designed, man-made environments? The way forward requires a firm factual and quantitative basis for describing the microbial potential in real environments, but this will not be achieved by either sequential monitoring or testing programmes. It requires imaginative conceptual thinking followed by validation of the proposed concepts. The presentation will evaluate the present status with respect to such an approach, considering the implications of both micro-scale mechanisms and molecular-scale control on macro-scale pollutant flux mediation.

The Pilot Plant Testing of the Continuous Extraction of Radionucleides using Immobilized Biomass Marios Tsezos, Ph.D., Department of Chemical Engineering, McMaster University, Hamilton, Ontario, Canada

The selective sequestering of metal ions from aqueous solutions by microbial biomass has been termed biosorption. Biosorption of metal ions is a phenomenon exhibited by both alive and dead microbial cells. The detailed investigation of the mechanism of biosorption has revealed that biosorption is a physical-chemical process whereby selected areas of the microbial cell exhibit high selectivity and specificity for the extraction and retention of specific metal ions from aquatic solutions.[1] This property is exhibited equally well or often better by dead cells than by the same cells alive. The use of proper chemical solutions (eluants) is capable of reversing the equilibrium of biosorption bringing the biosorbed metal ions back in solution and freeing the biomass active sites for subsequent reuse.[1]

Complex solution ionic matrices present a special challenge in so far as certain ions may compete with others during biosorption thus reducing the biomass metal uptake capacity.[2] Understanding of the mechanism by which coions can affect biosorption and the manipulation of corresponding solution parameters, like pH, can offset adverse coion effects.[1,2,3,4]

The small particle size of the microbial cell possesses however significant difficulties in the full scale industrial application of biosorption as a metal extraction and recovery process. The immobilization of the microbial biomass into particles of specified desirable physical and chemical properties alleviates this difficulty. Such a proprietary biomass immobilization process has been developed by the author. The produced immobilized biomass particles have been shown to have custom made particle size, high porosity and favorable biosorptive equilibrium and kinetic properties. A mathematical mass transfer kinetic

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3 Tsezos M., Nob S. H., Baird M.H.I., Biotech. & Bioeng., 32, 545, 1988

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model of biosorption has revealed the most significant engineering parameters that affect the immobilized biomass particle biosorptive behavior and has resulted in improving substantially the behavior of the produced biosorbent particles.[3]

Over the last 15 years, the detailed study of uranium biosorption has led to the design, construction and operation of two pilot plants for the continuous biosorptive recovery of uranium from the industrial biological leachates produced by Denison Mines in the Elliot Lake area of Canada[4]. The results from the two pilot plants have shown that the continuous biosorptive extraction and recovery of uranium is possible. The feed to the pilot plant has an incoming uranium concentration of 100 to 300 mg/L, a pH of 1.5 to 2.0 and a wide spectrum of cations and anions such as sulfates, calcium, iron, aluminum, rare earths etc. Some of these cations are present in concentrations as high as several thousand mg/L. The pilot plant produces a uranium concentrate stream with uranium concentrations as high as 14,000 mg/L.

An initial drop in the immobilized biomass uranium uptake capacity has been studied using techniques such as microprobe spectral analysis with digital image analysis and infrared spectroscopy. Recent results from the above work have suggested that the aluminum present in the biological leachate accumulates in the microbial biomass cell wall in the form of amorphous aluminum-silica hydrolysis products, resulting in a loss of uranium capacity. On the basis of the understanding of the mechanism of competition we have developed countermeasure strategies, the first of which has been tested with success.

The paper will present an overview of the above advances made a McMaster in the area of the biosorption fundamental and the development of the corresponding technology. This novel technology is a good example of the applications of biotechnology in the areas of metal recovery and environmental pollution control.

Research and Development Programs for Biological Hazardous Waste Treatment in the Netherlands Esther Soczo and Klaas Visscher, National Institute of Public Health and Environmental Protection, The Netherlands

Biotechnology is increasingly being applied in solving environmental problems in the Netherlands in the last decade. Micro-organisms have, of course, been used for centuries to process organic waste produced by man. Man has discovered that even non-naturally occurring substances such as polycyclic aromatic and chlorinated hydrocarbons can be degraded micro-biologically. The big advantage of biological hazardous waste treatment is that the pollutants are broken down into substances which are part of the natural cycle. This environmentally-friendly process can contribute to the recovery of our environment.

Biological treatment processes have been applied in the Netherlands in the cleaning of gases from industrial installations, in treating of waste water and in the cleanup of contaminated soil. The first biological filter for waste gas cleaning was developed in 1978. Since that time more than 200 biological filters have been installed. An other important Dutch development is the anaerobic waste water treatment. The design is being sold around the world. About 130 plants have been built between 1980 and 1988 inclusive, 90 of them abroad. Soil treatment is still a new area in the application of biotechnology. Landfarming methods and in-situ biorestoration have already been applied for the clean up of sites mainly contaminated with oil compounds. Bioreactors are in the development stage; the first pilot plants are being tested at this moment.

Environmental biotechnology is being promoted by the Dutch government within the framework of a number of subsidy programs. Altogether 23 million guilders (approx. $12 million) have been provided for environmental biotechnology projects in the past five years through a number of technology programs.

4.2 POSTER ABSTRACTS

PCR and LUX Constructions Bruce M. Applegate and C.M.B. Werner, Center for Environmental Biotechnology, The University of Tennessee, Knoxville

The lux transposon Tn 4431 has been used in constructing bioluminescent reporter strains. This technology has an important application in Environmental Biotechnology as bioluminescent reporters can be created for the biodegradation of polyaromatic hydrocarbons. The Polymerase Chain Reaction (PCR) was used in mapping the insertion site of the transposon Tn 4431 into the lower catabolic pathway of the naphthalene degrading plasmid pKA 1. Using primers for specific genes in the lower pathway of NAH7, a highly characterized naphthalene degrading plasmid whose catabolic genes show distinct homology with pKA 1, and primers for the insertion sequences of Tn 4431 it was possible to determine the insertion site of the transposon. This approach provided both the actual gene which was disrupted and the location of the transposon relevant to the transcription initiation site of the lower pathway.

Bioremediation Studies of a Coal Gasification Site Soil C. Baker and C. F. Kulpa, Ph.D., Department of Biological Sciences and the Center for Bioengineering and Pollution Control, University of Notre Dame

The bioremediation of wastes containing numerous and diverse components is most practically achieved by utilizing a mixed population of microorganisms whose members exhibit a variety of metabolic capabilities. The aim of this study is to examine the metabolic interactions among the members of a mixed microbial population enriched from a chemically complex waste site. Samples of soil from a coal gasification site were enriched with n-hexadecane or naphthalene, giving rise to two mixed cultures whose members demonstrated different metabolic properties. The ability of the n-hexadecane enriched culture to emulsify hydrocarbons was attributed to the presence of a surfactant producing organism identified as Acinetobacter calcoaceticus. Other members of the hexadecane enriched culture was comprised of at least two distinct Pseudomonas species, and other isolates thought to be Pseudomonas or Aeromonas species as well. Both cultures exhibited the ability to utilize naphthalene, though only one isolate from each culture was able to utilize naphthalene as a sole source of carbon in pure culture. To determine the effects of metabolites produced by the naphthalene utilizing organisms on the growth of the hexadecane enriched culture, growth was tested in minimal medium supplemented with cell-free medium from a two-week-old culture of the naphthalene enrichment which had been grown on 500 ppm naphthalene. Growth on this medium was only slight, but when hexadecane was included the culture grew readily, demonstrating no inhibitory effects of naphthalene breakdown products on the metabolism of hexadecane. An emulsification assay was employed to quantitate surfactant activity in microbial consortia. The implications of exploiting the emulsifying ability of one bacterial strain to facilitate better utilization of sparingly soluble polyaromatic hydrocarbons (PAHs) is addressed.

Plasmid and Catabolic Gene Frequencies in Linear Alkylbenzene Contaminated and Pristine Freshwater Ponds Alec Breen, Luis Jimenez, Ph.D., and Gary S.Sayler, Ph.D., Center for Environmental Biotechnology, The University of Tennessee, Knoxville and Thomas W. Federle, Ph.D., Environmental Safety Department, Procter and Gamble Co., Ivorydale Technical Center

A pond receiving high levels of linear alkylbenzene sulfonate (LAS) and a pristine pond were compared in their abilities to mineralize LAS. Individual isolates from these sites were surveyed for the presence of plasmid DNA and specific genes involved aromatic hydrocarbon oxidation. In general both LAS impacted and control sites demonstrated mineralization capabilities though the pristine site demonstrated a lag period relative to the contaminated site. Frequency of plasmid DNA was slightly higher at the control site (68%) than at the contaminated pond (44% in the weed bed and 51% in the pond water). Contaminated and control sites harbored similar frequencies of aromatic catabolic genes. These data suggest that the genetic potential for degradation of aromatic compounds, as assessed with these catabolic gene probes, is roughly the same and that acclimation to LAS is a key factor for LAS biodegradation. Enrichment cultures from the pond sites as well as activated sludge failed to yield any pure cultures capable of complete LAS degradation. This data supports the hypothesis that LAS mineralization is mediated by consortia rather than single organisms in environmental settings.

Biological Removal of Biochemical Oxygen Demand from a Boron Contaminated Industrial Waste Stream Kandi Brown and Janet Nichols, IT Corporation Biotechnology Applications Center

The objective of this investigation was to determine whether an industrial waste stream contaminated with 462 parts per million (ppm) boron was amenable to biodegradation to meet Publicly Owned Treatment Works (POTW) effluent criteria. The study employed a 5.0 liter (L) New Brunswick Scientific Bioflow III fermentation unit modified to recycle solids. The system was maintained at a biological solids retention time (BSRT) of 12 days with hydraulic retention time (HRT) set points ranging from 1 to 4 days. The fermentation unit was inoculated with 5.0 L of activated sludge and maintained at the following set points: 2 ppm dissolved oxygen, 7.0 - 8.0 pH, 500 revolutions per minute (rpm) agitation, and 25^{o}C temperature. The biochemical oxygen demand (BOD_5) of the influent feed stream was reduced by 98 percent to an effluent concentration of 15 ppm during operation at a 4 day HRT. The percent reduction of the influent BOD_5 concentration during operation at a 1 day HRT was 79 percent resulting in an effluent concentration of 202 ppm. The POTW effluent limit for BOD_5 was 550 ppm. Total organic carbon (TOC) and chemical oxygen demand (COD) removal efficiencies ranged from 98 to 64 percent, respectively, throughout the study. In conclusion, bioremediation was a viable technology to employ in the remediation of the influent waste stream. The study is currently moving to pilot scale.

Bioremediation of Hazardous Waste in a Slurry Reactor--The EIMCO Biolift Reactor Gunter Brox and Douglas E. Henify, EIMCO Process Equipment Company

Bioremediation in the slurry phase offers distinct advantages over in-situ treatment, land treatment, or composting: Better control of environmental conditions, i.e., pH, temperature, aeration, nutrients, desorption of contaminants into aqueous phase, and thus, more rapid treatment of certain wastes. A closed reactor allows volatile emission control and operation in aerobic or anaerobic mode. Gas recirculation of the off-gas back into the slurry by means of the diffusers allows biodegradation of the organic volatiles. Operation in a gas recirculation mode also allows oxygen enrichment and thus better oxygen transfer in the slurry. The addition of co-metabolites is possible as well. Potentially, gentically engineered bacteria will first be used in closed reactor systems.

The EIMCO Biofift Reactor^{TM} is a modified slurry agitator that uses a central airlift, bottom rakes, and an innovative diffuser design to achieve the basic objectives of mixing and aerating a slurry to sustain aerobic biodegradation processes. It can handle slurries of 25 - 50 vt% solids concentrations and provide mixing and aeration at a much lower energy consumption than conventional liquids/solids contact reactors. Several continuously fed, completely stirred reactors are often arranged in series to achieve optimum degradation kinetics and to meet low clean-up standards.

The Biolift^{TM} Reactor is being used in several RCRA and Superfund applications. A wide mix of organic contaminants have been degraded in different soil and sludge matrices.

A brief overview of other bioslurry reactors will be given and their developmental status will be discussed.

In Situ Depletion of Pentachlorophenol (PCP) from Contaminated soil by Phanerochaete spp. Diane M. Dietrich and Richard T. Lamar, Ph.D., Institute for Microbial and Biochemical Technology, US Forest Service Forest Products Laboratory

The purpose of this field study was to determine the ability of two white-rot fungi to deplete pentachlorophenol (PCP) from soil, that was contaminated with a commercial wood preservative. Inoculation of soil containing 250-400 ug g^{--1} PCP with either Phanerochaete chrysosporium or P. sordida resulted in an overall decrease of 88% to 91% of PCP in the soil in 6.5 wk. This decrease was achieved under suboptimal temperatures for the growth and activity of these fungi, and without the addition of inorganic nutrients. Since this soil had a very low organic matter content, peat was included as a source of organic carbon for fungal growth and activity. A small percentage (8% to 13%) of the decrease in the amount of PCP was a result of fungal methylation to pentachloroanisole (PCA). Gas chromatographic analysis of sample extracts did not reveal the presence of extractable transformation products other than PCA. Thus, if losses of PCP via mineralization and volatilization were negligible, as they were in laboratory-scale studies, most of the PCP was converted to nonextractable soil-bound products. The nature, stability, and toxicity of soil-bound transformation products, under a variety of conditions, must be elucidated before use of these fungi in soil remediation efforts can be considered a viable treatment technology.

Biolumninescent Sensing Technology Paul Dunbar, Center for Environmental Biotechnology, The University of Tennessee, Knoxville

Bacterial luminescence has shown to be a useful phenotype for laboratory research and potential for field studies. The various methods of bioluminescence measurements found in the literature include eyesight, 35 mm photographic film, X-ray film, and photon sensitive electronic equipment. Photomultipliers have been used primarily to monitor bioluminescence on-line in biological fermentors. Fiber optic sensors (or optrodes) allow in situ measurements which minimally disturb systems.

The combination of small optrodes and photomultipliers offer a potentially powerful tool for remotely sensing the bioluminescence of bacterial strains in complex environmental conditions. A photomultipler-optrode system has been constructed to measure the on-line light emission of bioluminescent strains in a simulated groundwater system.

Isolation and Characterization of mRNAs Transcribed by Catabolic Genes from Soil Sediment Microorganisms J.T. Fleming, Ph.D. and G.S. Sayler, Ph.D., Center for Environmental Biotechnology, The University of Tennessee, Knoxville

DNA extracted directly from complex environmental samples may be used to quantitatively determine the abundance of catabolic genes by nucleic acid hybridization. We sought to compliment DNA hybridization analysis with the isolation of catabolic mRNAs from solid microorganisms.

Sterile soil was inoculated with 10^8 and 10^9 cells/g soil from induced and uninduced cultures of Pseudomonas putita (PpG7) and subsequently total RNA was isolated by in situ lysis, phenol/choroform extraction and pelleting through CsCl. RNA samples electrophoresed on denaturing gels and stained with ethidium bromide displayed undegraded 16s and 23s ribosomal subunits after DNase treatment. Following transfer to nylon membranes, hybridization with ^{32}P-labeled nah ABCD probe and autoradiography, distinct mRNA bands unaffected by DNase digestion were observed with induced but not uninduced RNA.

An uninoculated Manufactured Gas Plant (MGP) soil contaminated with polyaromatic hydrocarbons containing 2×10^5 nah positive cells/g and a creosote contaminated soil containing 1×10^8 nah positive cells/g, both previously stored at 4^0 C, were warmed to room temperature for 2h in a water slurry and RNA was isolated as above. 10 ng/g of nah mRNA was obtained from the MGP soil as determined by RNA slot blots using PpG7 mRNA to make a standard curve. Northern blots of DNase treated creosote soil RNA probed with a ^{32}P-labeled in vitro transcribed nah ABCD RNA probe displayed discrete bands of similar mobility to PpG7 mRNA. These experiments suggest that in situ RNA isolation may be used to quantitate the expression of environmental catabolic genes.

Effect of Direct Surfactant Addition to Petroleum Biodegradation in Soil Undergoing Laboratory Scale Land Treatment Duane Graves and Maureen Leavitt IT Corporation, Biotechnology Applications Center

The direct application of surfactants to petroleum contaminated soil has been proposed as a mechanism to increase the bioavailability of insoluble compounds. Solubilization of hydrophobic compounds into the aqueous phase appears to be a significant rate limiting factor in petroleum biodegradation in soil. Nonionic surfactants have been developed to solubilize a variety of compounds, thus increasing the desorption of contaminants from the soil. In this study, laboratory treatability studies which emulate land treatment scenarios were used to monitor the bioremediation of petroleum contaminated soils. In efforts to achieve the lowest levels of residual petroleum hydrocarbons in the soil following biotreatment, 0.5 and 1.0% (volume/weight) surfactant was blended into soils under treatment. Two soil types were studied, a high clay content soil and a sandy, silty soil. In both cases, the addition of surfactant (Adsee 799^{O}, a blend of ethoxylated fatty acids) stimulated biological activity as indicated by increased heterotrophic colony forming units per gram of soil. However, the increased activity was not correlated with removal of petroleum hydrocarbons. In the sandy-silty soil, the amount of petroleum removed from the treatment was actually less than that removed in the surfactant-free treatment. In the clayey soil, no enhancement of petroleum hydrocarbon removal was observed in surfactant-amended treatments. The results suggest that the application of surfactants directly to the soil for the purpose of solubilizing hydrophobic compounds was not successful in achieving greater levels of petroleum hydrocarbon removal.

A High-Solids High-Yield Methanogenic Reactor: Microbial Biomass, Community Structure, Metabolic Status, and Activities David B. Hedrick, James B. Guckert, Ph.D., Institute for Applied Microbiology, The University of Tennessee, Knoxville, Brian Richards and William Jewell, Ph.D., Department of Agricultural and Biological Engineering, Cornell University, David C. White, M.D., Ph.D., Institute for Applied Microbiology, Department of Microbiology, The University of Tennessee, Knoxville, Environmental Sciences Divison, Oak Ridge National Laboratory

A novel semi-continuously fed anaerobic biomass digester operated at high solids (25%), thermophilic temperature (55 degrees C), and high pH (7.8 - 8.1), was found to be a very efficient system for the conversion of plant biomass to methane fuel. At 7.5 Lkg^{-1} day^{1}, significantly higher than any reported for particulate feedstocks. The organic loading rate was 24 gVS kg^{-1} day^{-1} at the highest productivity. Stable operation was observed for over 70 days. Best performance was observed with the less valuable, high C/N feedstocks. The biomass, community structure, and metabolic status of the high-solids system were determined over a feeding cycle by analysis of eubacterial and methanogenic membrane lipids. Microbial activities were determined by radiotracer analysis. Measures of biomass and community structure showed little variation over the feeding cycle, while measures of metabolic stress peaked at the disturbance of feeding and during starvation. Microbial activities, such as rate of acetate turnover and methane production rate, varied systematically over the feeding cycle. The evidence showed this system had selected for a microbial community capable of rapidly utilizing feedstock when provided, and surviving intermittent starvation. This high-solids anaerobic digester system is a viable candidate for a national biomass-to-energy system. The methods of analytical microbial ecology provide the insight required for transferring applications to production.

Treatability Studies To Develop Strategies For the Bioremediation of Tetrahydrofuran Contaminated Groundwater Ronald J. Hicks, Ph.D., Bioremediation Services, Groundwater Technology, Inc.

Industrial solvents, such as tetrahydrofuran (THF), are often found as contaminants in groundwaters due to accidental release or past disposal practices. Bioremediation represents a viable technology for the remediation of THF contaminated groundwaters. Possible strategies for implementing bioremediation technology for THF clean-up include the use of bioreactors, in-situ bioremediation, or a combination of these alternatives. Groundwaters, contaminated with THF at concentrations of between 200-1000 ppm, were collected and used in laboratory and pilot scale studies to identify and optimize possible bioremediation strategies. Biodegradation studies were performed to determine if indigenous bacteria could be effectively stimulated to degrade THF. To simulate in-situ bioremediation, THF contaminated groundwaters were amended with varying concentrations of nutrients and incubated under aerobic conditions. Greater than 99% of the THF was degraded within 120 hours in nutrient amended samples while less than 3% was removed in either unamended samples or poisoned controls. Bacteria capable of degrading THF were isolated and acclimated to increasing THF concentrations. These cultures were used as inocula in bench scale bioreactors to determine minimum retention times necessary for degradation of 400 ppm feed stocks. Respirometer studies were performed to optimize nutrient loading rates and for toxicity testing. Finally, engineering evaluations were performed to determine the hydrological and physical limitations to implementing in-situ bioremediation. The results of these studies were used to design a closed-loop system comprised of a bioreactor for treating recovered groundwaters coupled to a nutrient and oxygen infiltration system to stimulate indigenous THF degrading bacteria for in-situ treatment.

Mineralization of Linear Alkylbenzene Sulfonates by a Mixed Bacterial Culture

Luis Jimenez, Ph.D., Alec Breen, Gary S. Sayler, Ph.D., The Center for Environmental Biotechnology, The University of Tennessee, Knoxville and Thomas W. Federle, Ph.D., Environmental Safety Department, Procter and Gamble Co., Ivorydale Technical Center

Although Linear Alkyl Benzene Sulfonates (LAS) are biodegradable that does not mean that ultimate mineralization to CO_2 occurs. Previous studies have not reported mineralization of LAS by pure or mixed bacterial cultures. A bacterial consortium capable of LAS mineralization under aerobic conditions has been isolated from an activated sludge chemostat. Samples from the chemostat were plated on YEPG media for isolated colonies. Four different bacterial colonies were detected all Gram negatives rod-shaped bacteria which grow in pairs and short chains. Bacteria have shown morphological characteristics similar to the Pseudomonas spp. Based on the methylene blue active substances assay primary biodegradation of LAS was faster with the bacterial consortium than with the individual bacterial components. Bacterial colonies were grown together in minimal medium and ^{14}C-LAS as the only carbon source. After 13 days significant mineralization to $^{14}CO_2$ was obtained. Individual members of the bacterial consortium tested did not mineralize LAS. This study has shown that the four bacteria complemented each other and synergistically brought about a high rate of LAS mineralization suggesting a strong evidence for a degree of metabolic cooperation between the four bacterial components.

Competition and Simultaneous Maintenance of Polycyclic Aromatic Hydrocarbon and Chlorobiphenyl Degrading Bacteria in Continuous Culture Wade Johnston, Center for Environmental Biotechnology, The University of Tennessee, Knoxville

Continuous flow chemostat cultivation was used as a model system to investigate the simultaneous removal of mixed contaminants by defined mixed microbial populations, and to evaluate the co-maintenance of wild type and engineered strains involved in biodegradation. Experiments were conducted to determine the interactions among several wild type polycyclic aromatic hydrocarbon (PAH) and chlorobiophenyl degrading strains. A continuous culture system was constructed of wild type and/or engineered lux reporter stains capable of degrading naphthalene or 4-chlorobiphenyl as sole carbon sources. Bacterial population dynamics were monitored by growth on selective media. Naphthalene and 4-chlorobiphenyl degrading wild type strains showed simultaneous maintenance. At a dilution rate of 0.17 hr^{-1}, the naphthalene and 4-chlorobiphenyl degrading strains were maintained at 10^7 and 2×10^6 cells/ml respectively for 268 generations. When the dilution rate was raised to 1.71 hr^{-1}, the strains were maintained for an additional 604 generations.

A similar system containing the wild type naphthalene degrading strain 5R plus naphthalene degrading lux reporter strains was used. The wild type strain 5R, 5Rlux, and Pseudomonas putida RB1351lux were maintained at 10^8, 1.3×10^3, and 5×10^4 cells/ml respectively at a dilution rate of 0.51 hr^{-1} for 743 generations. At a dilution rate of 1.71 hr^{-1}, the 5R and P, putida RB1351lux strains were maintained as before for an additional 992 generations but 5Rlux was nearly washed out.

A system containing an engineered lux reporter strain (Ps. putida RB1351lux) and a community derived from a PAH contaminated soil was supplied with naphthalene and phenanthrene as well as a soil extract. The lux reporter strain was monitored by spread plating on selective media and was maintained at 2.3 - 2.8×10^6 cell ml^{-1} for 58 generations. Bioluminescence was maintained at 1.4 -2.3×10^{-8} amps. These results demonstrate that the lux strain can successfully compete against a wild type assemblage. The results demonstrate a useful chemostat approach for examining high order interactions among mixed populations promoting multiple contaminant removal.

Benzene, Toluene and Xylene Biodegradation Under Denitrifying Conditions in Soil Columns D.S. Kosson, Ph.D., G.F. Bowlen, B. J. Stuart, and P.D. Taylor, Department of Chemical & Biochemical Engineering, Rutgers University

A mixed microbial population was shown to simultaneously degrade toluene, m-xylene and p-xylene under anoxic conditions with nitrate as the electron acceptor. The columns were operated in an up-flow configuration, at an approximate velocity of 10 cm/day (200 ml) and only BTX (benzene, toluene, m-xylene, o-xylene and p-xylene) were simultaneously utilized as the carbon source. The soil column design allowed multiport sampling, with off-gas quantification and analysis. The complete transformation of up to 0.02 mM of each component was accompanied by production of N_2 and CO_2 and a concomitant drop in nitrate concentration. The toluene and m-xylene were removed before the first sampling port (14.3 cm) and the p-xylene was removed by the upper sampling ports (58.7 cm and 73.0 cm, respectively). Removal of the nitrate effectively stopped the transformation of the hydrocarbons.

The physical forces, dispersion and adsorption to soil, affecting hydrocarbon flow through the system were also investigated. The parameters affecting both dispersion and adsorption were fitted to a flow model by nonlinear regression. Dispersion within the flow system was quantified by ion tracer studies. Adsorption of the BTX compounds was quantified in batch serum bottle experiments, using multi-component mixtures to determine the adsorption coefficients.

The complete transformation of benzene and o-xylene was not observed during the experiments. Incomplete transformation of toluene and m-xylene was observed at 0.2 mM of each component.

Molecular Analysis of Manufactured Gas Plant Soils for Naphthalene Mineralization J. Sanseverino, Ph.D., C.M.B. Werner, J. Fleming, Ph.D., B.M. Applegate, J.M.H. King, Ph.D., G.S. Sayler, Ph.D. and J. Blackburn, Ph.D., Center for Environmental Biotechnology, The University of Tennessee, Knoxville

New molecular tools are being developed and tested to ascertain the biodegradability of hazardous waste by bacterial soil populations. The potential for manufactured gas plant (MGP) soil bacterial populations to degrade naphthalene was evaluated by the detection of the naphthalene genotype in direct DNA extracts of the soils and colony hybridization of cultured bacteria. The activity of the naphthalene-degrading populations was evaluated by $^{14}CO_2$ production from ^{14}C-naphthalene. Direct messenger RNA (mRNA) extraction from MGP soil was evaluated as an instantaneous measure of catabolic activity in MGP soil. The availability of naphthalene within the contaminated soils for bacterial degradation was assessed by measuring the bioluminescent response from a lux-naphthalene catabolic reporter plasmid, pUTK21.

DNA extracts of 5 MGP and 1 creosote-contaminated soil hybridized with a nahA gene probe indicating that the naphthalene genotype was present. ^{14}C-Naphthalene mineralization was observed with $^{14}CO_2$ rate constants ranging from 0.20 and 2.49h^{-1}. Phenanthrene, anthracene, and benz(a)pyrene were mineralized also by some of the soils. NAH7-related messenger RNA transcripts were detectable in the one MGP soil and one creosote-contaminated soil examined. Messenger RNA analysis of the remaining soils is in progress. Naphthalene bioavailability within the soil was indicated by a bioluminescent response from the lux-naphthalene construct. Nucleic acid extractions and hybridizations with gene probes allows a more rapid, more specific means than traditional kinetic and microbiological techniques of assessing the presence and activity of the PAH-degrading bacterial population in PAH contaminated soils.

A Rapid Method for Direct Extraction of DNA from Contaminated Soil and Sediments Yu-Li Tsai, Ph.D. and Betty H. Olson, Ph.D., Program in Social Ecology, The University of California

The combined techniques of DNA extraction from polluted environments and gene probe technology have become a useful tool to monitor particular organisms contributing to the bioremediation process. A rapid direct extraction of DNA from contaminated soil and sediments has been developed to overcome the disadvantages of previously published DNA extraction methods. The indigenous organisms contained in soil/sediment samples were lysed by lysozyme and 3 cycles of freeze-thawing in the presence of sodium dodecyl sulfate. The lysates were extracted with phenol-chloroform and followed by isopropanol precipitation to obtain crude total DNA. In addition to a high recovery rate (>90%), the yields of DNA were high (38 ug and 12 ug/g net wt. from sediments and soil, respectively). This method caused minimal shearing of the DNA. The crude DNA could be further purified by an Elutip - d column if necessary. This method has an additional advantage that only 1 g of sample is required, allowing the analysis of limited sample sizes and the processing of many samples in a relatively short period of time.

On-line, Real-time Biosensors for Bioprocesses in Microbial Consortia at the Institute for Applied Microbiology D.C. White, M.D. Ph.D., D.E. Nivens, M.J. Franklin, N.J.E. Dowling, Ph.D., T.J. Phelps, Ph.D., D.E. Hedrick, D.B. Ringelberg, Institute for Applied Microbiology, The University of Tennessee, Knoxville

Biofilms formed of consortia of microbes of different physiological types on surfaces are important in many processes of industrial import. IAM has developed on-line, non-destructive biomonitoring technologies and high resolution semi-continuous destructive monitoring techniques to follow adhesion, biofilm formation, community succession, nutritional status and metabolic activities. Primary work has been directed towards control of heat transfer resistance and the induction of microbially influenced corrosion (MIC) which is being increasingly recognized as an extremely important economic and safety problem for industrial water systems. The development of sufficiently rugged and accurate monitoring devices by which biofilm formation and activity of microbial biofilms can be monitored nondestructively, directly in water systems is the goal of this research. This on-line systems would allow the effective utilization of minimal levels of biocides and inhibitors as well as permit in situ testing of materials for MIC resistance. Several non-destructive technologies such as the quartz crystal microbalance (QCM), the attenuated total reflectance-Fourier transforming infrared spectrometer (ATR-FT/IR), and a genetically engineered bacterium containing the lux gene cassette (developed in collaboration with G.S. Sayler, of the UTK Center for Environmental Biotechnology) in which its bioluminescence can be used to define its presence on coupons are on-line devices which accurately measure biofilm formation. These may be correlated to the open circuit potential (OCP), which under specific conditions correlates with the formation of microbial biofilms and is a sufficiently rugged electrode for in situ use. Corrosion activity can be estimated by electrochemical impedance spectroscopy (EIS) which is non-destructive, correlates to microbial biofilm activity, is an accurate monitor of corrosion, can indicate localized (pitting) corrosion, and is also sufficiently rugged for in situ monitoring. Efforts are presently underway to apply these technologies to organic waste bioremediation and heavy metal immobilization by microbial consortia.

5 FINDINGS

An overall summary of the meeting was stated in the Preface of the published proceedings of the symposium.[5]

> The use of biotechnical processes in control of environmental pollution and in hazardous waste treatment is viewed as an advantageous alternative or adduct to physical chemical treatment technologies. Yet, the development and implementation of both conventional and advanced biotechnologies in efficacious field applications suffer from numerous technical, regulatory and societal uncertainties.
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> With the application of modern molecular biology and genetic engineering, there is clear potential for biotechnical developments that will lead to breakthroughs in controlled and optimized hazardous waste treatment for *in situ* and unit process use. There is, however, great concern that the fundamental research base may not be able to sustain continued technology development.
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> Some of these issues have been discussed in a fragmented fashion within the research and development community. A basic research agenda has been established to promote a sustainable cross-disciplinary technology base. This agenda includes developing new and improved strains for biodegradation, improving bioanalytical methods to measure strain and biodegradation performance, and providing an integrated environmental and reactor systems analysis approach for process control and optimization.
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> There remains an identified need to promote cross-disciplinary communication of technology development and application, and to identify choke points that impinge on the effective commercial application of the technology. For these reasons, industrial, federal, and academic partners joined together to sponsor this current dialogue on moving modern environmental biotechnology from the laboratory to successful field application. Unlike other efforts to communicate the technology, this symposium was planned to not only identify current practices and state-of-the-art, but also to identify perceptional and regulatory issues that affect credible applications and evaluation of the technology. In this regard, we must acknowledge the concerned foresight of the sponsors of the symposium, International Technology Corporation; the American Cyanamid Company; the U.S. Air Force Office of Scientific Research; the University of Tennessee; Waste

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5 Sayler, G.S., R.D. Fox, and J. W. Blackburn, Eds., 1991, Environmental Biotechnology for Waste Treatment, Plenum Press, New York.

Management Research and Education Institute; support from the Oak Ridge Waste Management Association; the planning and steering committee and the symposium participants.

A goal of the symposium was to communicate a broad view of environmental biotechnology ranging from conventional practices in biological waste treatment to genetic engineering perspectives in *in situ* treatment technology. From the beginning it was acknowledged that the biology was intimately linked to the environmental application and the engineering design in implementing the technology. This major scale-up consideration is the critical technical hurdle in moving the technology from the lab to practical field use. In this scale-up, there are major limitations in monitoring and controlling biotechnical processes, and these limitations further confound societal and regulatory perception of the credibility of the technology.

The outcome of this symposium contributes to identifying applications of fundamental research in emerging technology and to defining industrial research needs. It is also anticipated that strategies will be forthcoming to overcome concerns of the safety and efficacy of the technology. There appear to be numerous opportunities for environmental biotechnology to contribute to integrated waste management, but care must be taken to demonstrate reliable technology in order to capitalize on these opportunities.